New Phytol. (1996), **133**, 253–260

The roles of internal iron hydroxide precipitation, sulphide toxicity and oxidizing ability in the survival of *Stratiotes aloides* roots at different iron concentrations in sediment pore water

BY A. J. P. SMOLDERS AND J. G. M. ROELOFS

Department of Ecology, Research Group of Environmental Biology, University of Nijmegen, P.O. Box 9010, NL-6500 GL Nijmegen, The Netherlands

(Received 15 November 1995; accepted 4 January 1996)

SUMMARY

Stratiotes aloides L. is an aquatic macrophyte that occurs in waters on reduced peaty sediments which have a relatively narrow range of free-iron content in the sediment.

Comparison of different aquatic macrophytes reveals that species from reducing sediments have much lower oxidizing ability than do species from oxidizing sediments. Compared with those other species from reducing sediments, the oxidizing ability of *Stratiotes aloides* is very low and probably makes the species very vulnerable to sulphide toxicity and internal precipitation of iron hydroxide.

Apoplastic iron contents were determined for *Stratiotes* roots growing in sediments with different free-iron concentrations. Roots collected from sediment with a low free-iron content appeared to have a relatively low apoplastic-iron content whereas roots from sediments with a relatively high free-iron concentration had a relatively high apoplastic-iron content. In sediments with low free-iron levels, sulphide levels are generally high.

Using light microscopy, iron hydroxide precipitates were observed around the endodermis and cortical air spaces of the roots of *Stratiotes aloides* growing in iron-rich sediments. Internal iron oxidation outside the endodermis can prevent iron toxicity inside the stele and thus enable root apices to survive. The root hairs, however, die because of iron hydroxide precipitation at their base; the dead roots have the highest visible iron hydroxide content. Excessive internal iron hydroxide precipitation and the consequential early death of the roots probably explains the absence of *Stratiotes aloides* in locations with high free-iron levels in sediment pore water.

Key words: Iron plaque and toxicity, *Stratiotes aloides* L., radial oxygen loss, oxidizing activity, sulphide toxicity.

INTRODUCTION

Stratiotes aloides L. is a floating plant species distributed mainly in waters on reduced peaty sediments. The apical and subapical parts of the roots, which are normally covered with root hairs, penetrate the sediment and enable the plants to take up nutrients from it. In the Netherlands, healthy *Stratiotes* strands are restricted to waters with a relatively narrow range of free-iron content in the sediment (De Lyon & Roelofs, 1986). These free-iron concentrations are rarely less than 50 μmol l^{-1} or greater than 800 μmol l^{-1}. The reasons for this relatively narrow range are unknown, but observations reveal that sulphide and iron toxicity might be involved.

At present, due to agricultural land-use, groundwater levels have decreased considerably in large parts of the Netherlands. As a result, seepage has decreased markedly in many areas, and alkaline, sulphate-enriched water from the river Rhine is allowed in to prevent these areas from drying out in summer. Owing to iron sulphide precipitation resulting from greater reduction of sulphate and decreased iron input, caused by decreased seepage, iron levels have become very low in many peaty sediments in the Netherlands (Smolders & Roelofs, 1993). In the *Stratiotes* stands where levels of dissolved iron are low, the apical parts that penetrate into the sediment are often flaccid, and the sediments smell of hydrogen sulphide. Sulphide toxicity, which is known to affect the roots of aquatic and semi-

aquatic plants (Allam & Hollis, 1972; Yoshida & Tadano, 1978; Koch & Mendelssohn, 1989), might play a role in the observed decreased of root vitality in sediments with very low concentrations of free iron.

Iron toxicity might play a role in locations where there is a high level of dissolved iron in sediment pore water. Excessive uptake and oxidation of ferrous iron in living tissues can result in the production of highly toxic hydroxyl radicals (Hendry & Brocklebank, 1985; Bienfait, 1989). Aquatic and semi-aquatic species are therefore forced either to tolerate increased ferrous iron levels insider their cells, or to avoid iron toxicity through the exclusion of ferric iron from their roots (Bienfait, 1989). Oxidation of the rhizosphere by leakage of oxygen, and the consequential oxidation and precipitation of iron hydroxide outside the root or on the root surface of waterlogged plants is generally regarded as a mechanism by which iron toxicity is avoided (Bartlett, 1961; Armstrong & Boatman, 1967; Armstrong, 1978; Yoshida & Tadano, 1978; Rozema, Luppes & Broekman, 1985; Laan *et al.*, 1989; Laan, Smolders & Blom, 1991; St-Cyr, Fortin & Campbell, 1993).

More, specifically, iron(II) exclusion by the roots is thought to be determined by the equilibrium between the oxidation and reduction processes in the rhizosphere. This equilibrium depends on the oxidizing ability of the roots on the one hand and the reductivity of the sediment on the other (Taylor, Crowder & Rodden, 1984). As long as plants are able to oxidize the immediate surroundings of the roots, iron will precipitate predominantly outside the roots. Thus, species with high oxidizing abilities have high iron-excluding capacities (Bartlett, 1961; Yoshida & Tadano, 1978) and often show iron hydroxide precipitations on the root surface (Bartlett, 1961; Armstrong & Boatman, 1967; Green & Etherington, 1977; Chen, Dixon & Turner, 1980; Taylor *et al.*, 1984; Laan *et al.*, 1989; Laan, Smolders & Blom, 1991; St-Cyr *et al.*, 1993). The amount of radial oxygen loss from the roots of a species seems to depend on the reductivity of the sediment on which the species occurs (Smits *et al.*, 1990). Isoetid species (which occur on oxidative sediments) are well known for their relatively high radial losses of oxygen (Wium-Andersen & Andersen, 1972; Tessenow & Baynes, 1978; Smits *et al.*, 1990).

This paper presents results of a study on the role of both sulphide and iron on the vitality of the roots of *Stratiotes aloides* under different iron levels on the sediment. We also quantify the oxidative ability of *S. aloides* and some other aquatic macrophytes from reducing and oxidizing sediments in order to obtain insight into the role of the oxidizing ability of the roots in susceptibility to sulphide and iron toxicity.

MATERIALS AND METHODS

Free-iron/sulphide interactions

To establish the possible relationship between iron and sulphide levels, samples were collected from 75 different surface waters in the peaty lowland areas of the Netherlands during the summers of 1992 and 1993. The samples were sucked from the upper 10 cm using porous ceramic cups which were connected to vacuum infusive flasks by means of airtight tubes (lysimeters). Iron and sulphide were analysed as described by Roelofs (1991).

Observations on Stratiotes aloides *roots collected in the field*

In August 1993 mature *Stratiotes* plants with a rosette diameter of ± 25 cm were collected in four different locations in the Netherlands. All locations had peaty sediments and were selected for their different free-iron levels in sediment pore water. We selected a ditch near Glimmen in the province of Groningen, a ditch near Tienhoven in the province of Utrecht, a ditch near Zegveld in the province of Zuid-Holland and an artificial pond in the grounds of the University of Nijmegen. From each of these, sediment pore water samples were collected and analysed.

Cross sections of fresh roots were cut with a razor blade and studied under a light microscope. With the aid of a tetrazolium-staining method (Bertani, Brambilla & Menegus, 1981) different parts of the roots were checked for blue coloration which, in other experiments, has proved to be a good measure of the viability of the tissues. Furthermore, the root surfaces were carefully checked for the presence of iron hydroxide precipitates.

Sub-apical parts of the roots of 12 plants from each location were carefully cut, and rinsed with demineralized water. Only roots which still possessed a healthy-looking apex were selected. Next, the extracellular iron was extracted with sodium dithionite and determined with a bipyridyl solution, as described by Bienfait, van den Briel & Mesland-Mul (1984) and Laan *et al.* (1989). Because a high concentration of apoplastic iron was expected, 0·25 g of fresh root material was used and the concentration of the bipyridyl solution was increased to 4·5 mM. The extraction time was extended to $\pm 1{\cdot}5$ h. From each plant four roots were selected, analysed separately and the results averaged per plant. From the plants from the ditch near Zegveld, however, very few intact roots could be obtained and one root was selected per plant. After extraction, the root parts were dried at 70 °C and weighted.

The iron content of the shoots was determined for leaves of intermediate age, according to Smolders & Roelofs (1993).

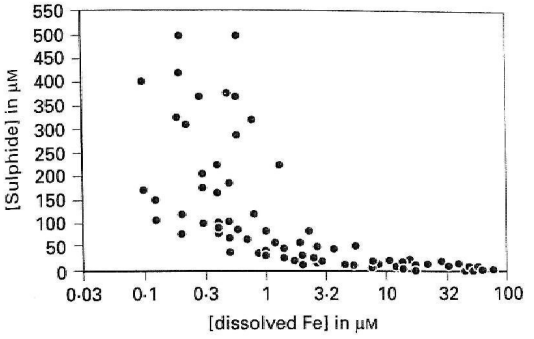

Figure 1. Relationship between sulphide and free-iron levels in sediment pore water samples collected in 75 locations in the Netherlands with reduced organic sediment.

Methylene blue oxidizing ability

Oxidizing ability of the roots of *S. aloides* and some aquatic macrophytes from reducing and oxidizing sediments were studied according to the methylene blue method described by Laan *et al.* (1989) in a cuvette consisting of a root and a shoot compartment. The shoot compartment was filled with water similar to water from which the plants were collected in the field, bubbled with air, and illuminated (180 μmol $m^{-2} s^{-1}$). The plants were collected in the field and investigated immediately for the detection of the methylene-blue oxidizing ability. This was defined as the oxidation of leuco-methylene blue in nmol $cm^{-1} h^{-1}$ calculated after 12 h of incubation of root systems in a 0·1 % (w/w) agar solution containing 0·04 g of methylene blue (Laan *et al.*, 1989). The methylene blue oxidizing ability calculated is the mean value for the parts of the roots that showed methylene blue oxidizing ability.

Sulphide toxicity

Mature plants of *S. aloides* with a rosette diameter of ±25 cm were collected in artificial ponds on the grounds of the University of Nijmegen and placed in aquaria (8 h dark, 16 h light at 180 μmol $m^{-2} h^{-1}$) with nutrient levels according to Smolders, Den Hartog & Roelofs (1995). The apical 15 cm of each root was carefully put through a hole in the rubber septum of a screw-cap and sealed with plasticine to prevent leaks. A blackened serum bottle containing solution in which the nutrient levels were the same as in the aquaria was attached to the screw cap. The contents of the serum bottles had been flushed with nitrogen to make them anoxic. Different amounts of sulphide were added in order to obtain a staged sulphide gradient of 0, 5, 10, 25, 50, 100, 250, 500 and 1000 μmol l^{-1}. The pH of the medium in the serum bottles was adjusted to pH 7 by adding hydrochloric acid. The media in the serum bottles were changed daily, the water in the aquaria continuously. Sulphide levels in the serum bottles were checked before and after changing. The roots were checked for viability twice per day. Dead roots were easily distinguished by their flaccid appearance which was used as the criterion for viability. For each concentration 20 roots were used.

RESULTS

Relationship between free-iron and sulphide levels in sediment pore water

In the sediment pore water samples obtained from the peaty lowland areas of the Netherlands, iron and sulphide concentrations appear to be inversely related (Fig. 1). In sediments with iron levels higher than 50 μmol l^{-1} sulphide levels were very low. In

Table 1. (*a*) *Apoplastic iron concentrations in the roots, and iron contents of leaves of intermediate age, of* Stratiotes aloides *plants collected in four different locations in the Netherlands* (n = 12). (*b*) *Mean values for some important chemical parameters of sediment pore water* (n = 5)

	Artificial pond, University	Glimmen	Tienhoven	Zegveld
(*a*) Iron contents				
Apoplastic iron (μmol g^{-1} d. wt)	77·1 ± 24·5	68·1 ± 29·1	11·3 ± 10·2	0·7 ± 0·4
Iron in shoot (μmol g^{-1} d. wt)	18·7 ± 6·8	21·0 ± 7·2	5·7 ± 1·0	0·1 ± 0·1
(*b*) Sediment pore water				
Iron (μmol l^{-1})	418 ± 95	555 ± 161	89 ± 27	1·1 ± 1·3
Sulphide (μmol l^{-1})	< 0·1	< 0·1	< 0·1	24·7 ± 8·8
pH	6·9 ± 0·1	7·1 ± 0·3	6·7 ± 0·2	7·3 ± 0·1
Bicarbonate (μmol l^{-1})	1598 ± 112	3111 ± 551	3096 ± 620	4786 ± 712
Ortho-phosphate (μmol l^{-1})	1·9 ± 0·5	4·1 ± 0·7	8·0 ± 3·0	25·1 ± 6·8
Manganese (μmol l^{-1})	14·5 ± 2·1	19·8 ± 3·9	10·8 ± 4·1	6·5 ± 2·1
Calcium (μmol l^{-1})	810 ± 187	1313 ± 272	1712 ± 353	2531 ± 510
Chloride (μmol l^{-1})	620 ± 69	570 ± 56	1078 ± 230	4434 ± 650

Mean values ± SD are given.

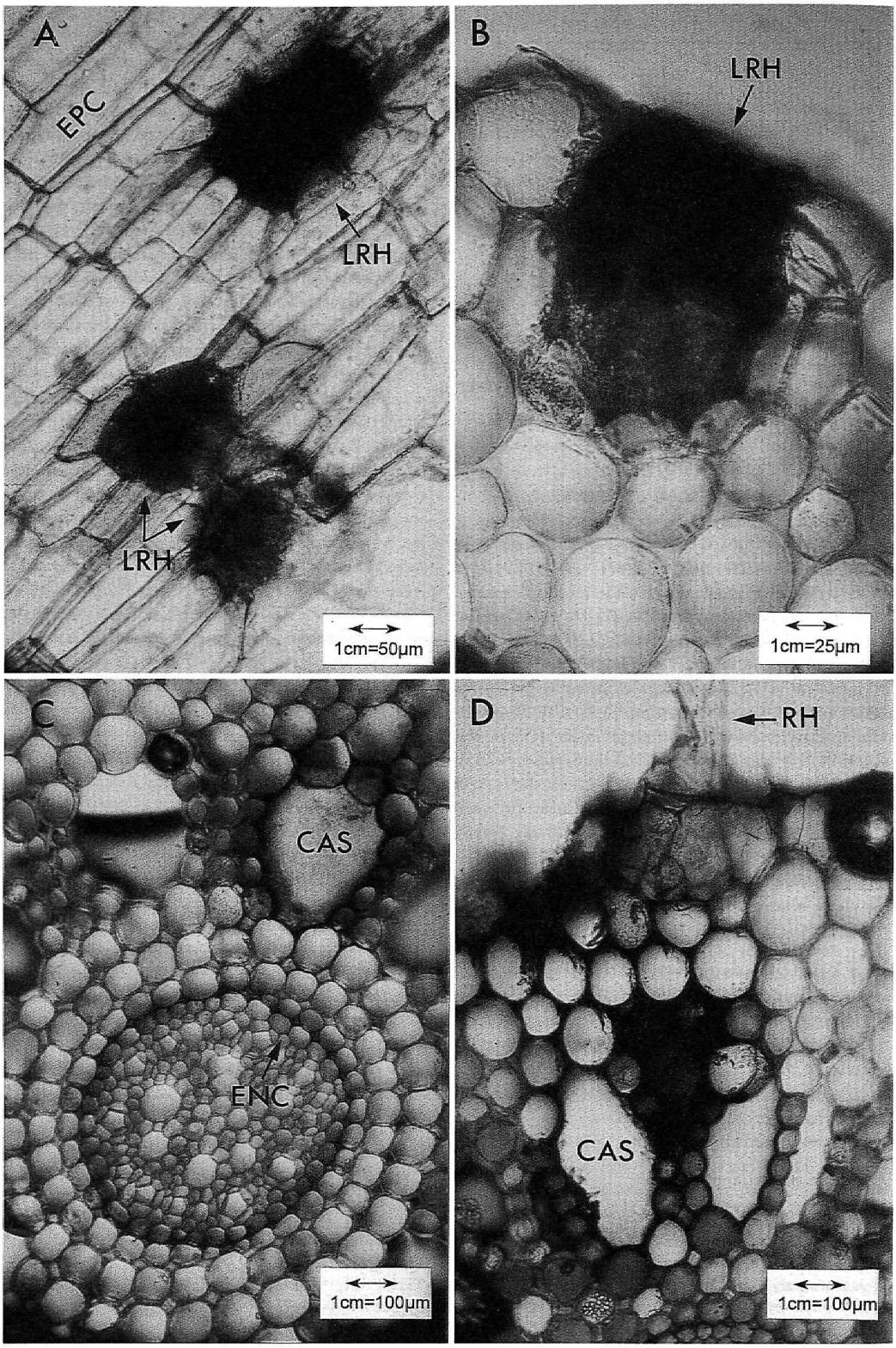

Figure 2. For legend see opposite.

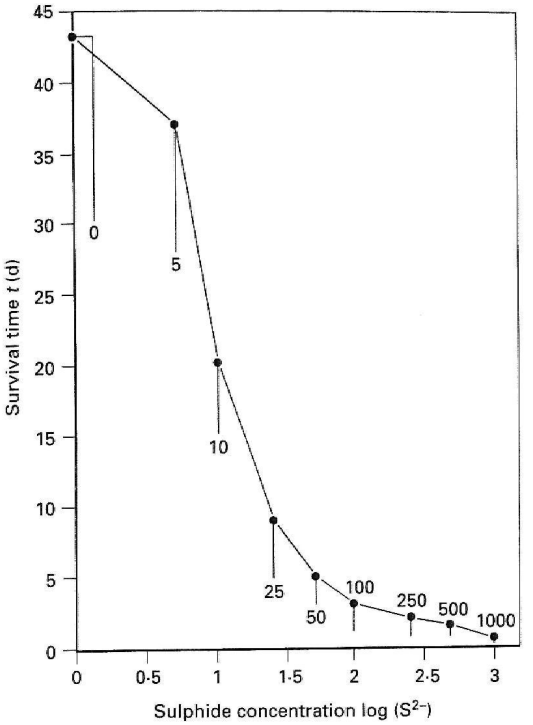

Figure 3. The effects of different sulphide concentrations on the survival of *Stratiotes aloides* roots. The points indicate the times after onset of the experiments at which 80 % of the roots had died. The vertical lines under the points indicate the time span in which roots died during the experiment. The values under the vertical lines indicate the absolute sulphide concentrations in μmol l^{-1}.

sediments where free-iron levels are lower than 20 μmol l^{-1}, sulphide levels were much higher.

Observations on the Stratiotes *roots from the different locations*

Apoplastic free-iron levels were very low in the roots from the ditch near Zegveld and much higher in those from the artificial pond at the University grounds and those from the ditch near Glimmen (Table 1), the values for roots collected near Tienhoven being intermediate. These differences correspond to the free-iron levels in sediment pore water which were high in the artificial pond and in the ditch near Glimmen, low in the ditch near Zegveld and intermediate in the ditch near Tienhoven. Sulphide could only be detected in the ditch near Zegveld. The very low free-iron levels in the ditch near Zegveld corresponded to very low iron levels in the shoots of the plants which were chlorotic. The iron content of the shoots was highest in those from the artificial pond and those from the ditch near Glimmen, and intermediate in those from the ditch near Tienhoven. Typical symptoms indicating iron toxicity (i.e. brown necrotic spots on the leaves; Laan *et al.*, 1991) were not observed. Some other nutrient concentrations of the sediment pore water are also given in Table 1.

The root surfaces of the plants collected from the artificial pond in the University grounds and from the ditch near Glimmen were speckled with red spots. Observation with the light microscope revealed that these were located where root hairs had been attached. Nearly all root hairs were decayed; few intact ones remained. External iron hydroxide precipitates were only found on the spots where root hairs had decayed (Fig. 2*a*). Cross sections of these roots revealed that iron hydroxide precipitates were found throughout the cortex but especially around the air spaces lying in line with decayed root hairs (Fig. 2*b*, *d*). Around the endodermis of all roots a clear reddish-brown ring of iron hydroxide was visible (Fig. 2*c*).

In roots that still had an apex the intensity of iron hydroxide deposition varied between moderate and heavy around the endodermis as well as around the cortical air spaces and the bases of (mostly decayed) root hairs (Fig. 2*b*). Large parts of the cortex of these roots did not show any coloration in the tetrazolium test, indicating that these parts might have been dead. The stele tissue of these roots, however, still showed coloration indicating that, next to the apex at least, it was more or less intact. Many of the roots had lost their natural flexibility and broke very easily. In these roots only very heavy iron hydroxide precipitations were found, and stele tissue showed little or no coloration in the tetrazolium test.

The roots of the plants collected from the ditch near Zegveld did not show any visible iron hydroxide precipitation. These plants, however, possessed only a few living roots which penetrated the sediment. From the sediment, the remnants of roots which were obtained were mainly flacid. The vital roots that could be obtained all showed a clear blue coloration in the tetrazolium test. No iron hydroxide precipitation could be observed.

The plants from Tienhoven had many healthy-looking, live roots with ample root hairs. No brittle roots were found. External iron hydroxide precipi-

Figure 2. (*a*) Surface of *Stratiotes aloides* roots showing the absence of iron hydroxide precipitation on major parts of the root surface. Only the spots where root hairs had been attached show iron oxide precipitates. (*b*) Cross section through a root of *S. aloides* showing iron hydroxide precipitation at the base of a decayed root hair. (*c*), (*d*). Cross sections through a root of *S. aloides*, showing iron hydroxide precipitation around the endodermis and cortical air spaces. EPC, epidermal cell; LRH, location of former root hair; ENC, endodermal cell; CAS, cortical air space; RH, remainder of root hair.

Table 2. (*a*) *Mean methylene-blue oxidizing abilities of various species.* (*b*) *Mean values of some physico-chemical parameters of the sediments and water layer**

	Species from oxidizing sediments	Species from reducing sediments	*Stratiotes aloides*
(*a*) Number of species	5†	6‡	1
Methylene blue oxidizing ability (nmol cm^{-1} h^{-1})	129 (85–148)	32 (25–39)	6·0
(*b*) Redox potential (mV)	71 (1·5–167)	−160 (−170 to −149)	−175
Organic matter (% of d. wt)	4·8 (2·0–7·0)	20 (8–35)	30
Iron (S) (μM)	793 (230–1126)	706 (290–1076)	298
Phosphate (S) (μM)	7·9 (5·0–11)	3327 (12–46)	—
Phosphate (W) (μM)	0·8 (0·3–1·4)	2·8 (1·2–6·1)	3·7
Leakage pattern	Along the whole length of the roots	Along apical parts of the roots	Along apical parts of the roots

* These values are obtained from De Lyon & Roelofs (1986). The range of the mean values for every species is given in parentheses. S, sediment; W, in water layer.

† Five isoetid species from oxidizing sandy sediment. (*Echinodorus ranunculoides* Engelm., *Pilularia globulifera* L., *Littorella uniflora* (L.) Ascherson, *Luronium natans* (L.) Rafin. and *Eleocharis acicularis* (L.) R. et Sch.)

‡ Six species from organic reducing sediment (*Potamogeton compressus* L., *Ranunculus. circinatus* Sibth., *Myriophyllum verticillatum* L., *Glyceria maxima* (Hartman) Holmberg, *Nymphoides peltata* (Gmel.) O. Kuntze, *Nymphaea alba* L.).

tations were only found at spots where root hairs had decayed. Only moderate iron hydroxide precipitations were observed around the endodermis and the cortical air spaces.

Sulphide toxicity in Stratiotes *roots*

Figure 3 shows the susceptibility of the roots of *S. aloides* at the different sulphide concentrations in the laboratory experiment. The survival of the roots appears to be seriously affected by sulphide levels higher than 5 μmol l^{-1}.

Methylene-blue oxidizing ability

Aquatic macrophytes from reducting sediments have considerably lower methylene-blue oxidizing ability than do plants from oxidative sediments (Table 2). Compared with other species from reducing sediments, *S. aloides* has an even lower methylene-blue oxidizing ability. Furthermore, the plants from the oxidative sediments show methylene-blue oxidation along the entire length of their roots, whereas the plants from the reductive sediments only show methylene-blue oxidizing ability along the apical parts of their roots. Mean nutrient levels and organic matter concentrations in the sediments and the water layer are lower for isoetid species, from the oxidizing sediments than for the species from the reductive sediments (Table 2).

DISCUSSION

In general, aquatic macrophytes from oxidizing sediments, i.e. sandy sediments with a relatively high redox potential have a high oxidizing ability (Table 2) and leak oxygen along the entire length of the root. This indicates that they do not possess morphological adaptations to prevent radial losses of oxygen (Armstrong, 1978; Laan *et al.*, 1989; Smits *et al.*, 1990). Dense stands of these species are known to increase the redox potential of the sediment considerably (Wium-Andersen & Andersen, 1972; Tessenow & Baynes, 1978). Aeration of the sediment probably increases mineralization of organic matter and thus the availability of nutrients and CO_2, which are taken up by the roots (Sondergaard & Sand-Jensen, 1979; Roelofs, Schuurkes & Smits, 1984). Aeration also enhances nitrification, and thus nitrate availability, which is important, since these species use nitrate as their nitrogen sources (Schuurkes, Kok & Den Hartog, 1986).

In general, species from organic reducing sediments have lower oxidizing abilities than species from oxidizing sediments and only leak oxygen from the (sub-)apical parts of the roots (Table 2; Smits *et al.*, 1990). In these species, suberization or lignification of the outer cortex prevents radical oxygen losses along the basal parts of the roots, thereby permitting the aeration of the vital apex (Armstrong, 1978) where, since the oxygen sink of the reduced sediments is very strong, the tissues would otherwise become lower than the critical levels of oxygen for metabolic processes (Armstrong & Webb, 1985). Furthermore, the prevention of oxygen leakage along large parts of the roots prevents the build-up of aerobes. Aerobes diminish the size of the oxidized rhizosphere and thus increase the entry of phytotoxins (Armstrong, Brandle & Jackson, 1994). Of course, prevention of radial losses of oxygen will also hinder the uptake of nutrients from the sediments, but the availability of nutrients is generally higher in

the reduced organic sediments (Table 2). Finally, nutrient uptake by the roots might be far less important for species from reduced sediments than for species from oxidizing sediments, as nutrient concentrations in the water layer above the former are generally much higher than in the latter (de Lyon & Roelofs, 1986).

The oxidizing ability of *Stratiotes aloides* is much lower than that of the other species from reducing sediments which have been studied (Table 2). This could, at least partly, be caused by the very long roots of the species (more than 1 m) which will cause a relatively high resistance to the longitudinal diffusion of oxygen through the roots. Oxidation by the rhizosphere is thought to be important in the detoxification of Phytotoxins such as sulphide and Fe(II) (Armstrong, 1978; Bienfait, 1989; Armstrong *et al.*, 1994), and therefore the susceptibility of the roots to sulphide and iron (II), is probably caused by their very low oxidative ability. However, the sensitivity to sulphide might be overestimated in our experiment because circulation currents in the water culture will hinder re-oxidation of the rhizosphere.

We have shown that iron oxidation takes place in the root cortex of *Stratiotes aloides*. This agrees with the findings of Taylor *et al.* (1984) who hypothesized that in species with very low oxidizing abilities iron oxidation would occur primarily inside the roots. The amount of iron precipitated in the roots depends mainly on the iron levels in sediment pore water (Table 1). Symptoms indicating iron toxicity, such as brown necrotic leaf spots, could not be detected in *S. aloides*. Apparently excessive uptake of Fe(II) is prevented by its oxidation in the cortex, and the premature death of the root hairs and the roots. Furthermore, the endodermis seems to function as an effective barrier to excessive iron uptake.

It appears that iron enters the roots via the root hairs. However, as no iron precipitates could be observed inside the stele, and as the tetrazolium stainings revealed that, in the roots with a living root apex, stele tissue was still alive, the endodermis of *Stratiotes aloides* seems to prevent, or at least delay, attainment of toxic iron levels inside the stele. In this way the survival of the apex, vital for the survival of the root as a whole, is prolonged, as sugars and other metabolites can only be transported to the apex by living phloem tissue. Although cortical iron oxidation might prolong the lifetime of the roots when iron levels are high, we observed that most of the roots with dense deposits of iron hydroxide were dead. Furthermore, the root hairs appeared to decay early, probably because of iron hydroxide precipitation at their base.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. dr. C. den Hartog, Dr P. Laan, Mr P. van Avesaath and Mr B. Kelleher for critically reading earlier versions of the manuscript, and Mr J. van Dijk for practical assistance.

REFERENCES

Allam AI, Hollis JP. 1972. Sulphide inhibition of oxidases in rice roots. *Phytopathology* **62**: 634–639.

Armstrong W. 1978. Root aeration in the wetland condition. In: Hook DD, Crawford RMM, eds. *Plant Life in Anaerobic Environments*. Michigan: Ann Arbor, 269–297.

Armstrong W, Boatman DJ. 1967. Some field observations relating the growth of bog plants to conditions of soil aeration. *Journal of Ecology* **55**: 101–110.

Armstrong W, Brandle P, Jackson MB. 1994. Mechanisms of flood tolerance in plants. *Acta Botanica Neerlandica* **43**: 307–358.

Armstrong W, Webb T. 1985. A critical oxygen pressure for root extension in rice. *Journal of Experimental Botany* **36**: 1573–1582.

Bartlett RJ. 1961. Iron oxidation proximate to plant roots. *Soil Science* **92**: 372–379.

Bertani A, Brambilla I, Menegus F. 1981. Effect of anaerobiosis on carbohydrate content in rice roots. *Biochemie und Physiologie der Pflanzen* **176**: 835–840.

Bienfait HF. 1989. Prevention of iron stress in iron metabolism of plants. *Acta Botanica Neerlandica* **38**: 105–129.

Bienfait HF, van den Briel W, Mesland-Mul NT. 1984. Free space iron pools in roots. *Plant Physiology* **78**: 596–600.

Chen CC, Dixon JB, Turner FT. 1980. Iron coatings on rice roots: morphology and models of development. *Soil Science Society of America Proceedings* **44**: 1113–1119.

De Lyon MJH, Roelofs JGM. 1986. *Aquatic macrophytes in relation to water quality and sediment characteristics*. The Netherlands: Department of Ecology, University of Nijmegen. (In Dutch.)

Green MS, Etherington JR. 1977. Oxidation of ferrous iron by rice (*Oryza sativa* L.) roots: a mechanism for waterlogging tolerance? *Journal of Experimental Botany* **28**: 678–690.

Hendry GAF, Brocklebank KJ. 1985. Iron-induced oxygen radical metabolism in waterlogged plants. *New Phytologist* **101**: 199–206.

Koch MS, Mendelssohn IA. 1989. Sulphide as a soil phytotoxin: differential responses in two marsh species. *Journal of Ecology* **77**: 565–578.

Laan P, Smolders A, Blom CWPM, Armstrong W. 1989. The relative roles of internal aeration, radial oxygen losses, iron exclusion and nutrient balance in flood-tolerance of *Rumex* species. *Acta Botanica Neerlandica* **38**: 131–145.

Laan P, Smolders A, Blom CWPM. 1991. The relative importance of anaerobiosis and high iron levels in flood-tolerance of *Rumex* species. *Plant and Soil* **136**: 153–161.

Roelofs JGM. 1991. Inlet of alkaline river water into peaty lowlands: effects on water quality and *Stratiotes aloides* L. stands. *Aquatic Botany* **39**: 267–293.

Roelofs JGM, Schuurkes JAAR, Smits AJM. 1984. Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. *Aquatic Botany* **18**: 389–411.

Rozema J, Luppes E, Broekman RA. 1985. Differential responses of salt marsh species to variation of iron and manganese. *Vegetatio* **62**: 293–301.

Schuurkes JAAR, Kok CJ, Den Hartog C. 1986. Ammonium and nitrate uptake by aquatic macrophytes from poorly buffered and acidified waters. *Aquatic Botany* **24**: 131–146.

Smits AJM, Laan P, Thier RH, van der Velde G. 1990. Root aerenchyma, oxygen leakage patterns and alcoholic fermentation ability of the roots of some nymphaeid and isoetid macrophytes in relation to the sediment type of their habitat. *Aquatic Botany* **38**: 3–17.

Smolders A, Den Hartog C, Roelofs JGM. 1995. Germination and seedling development in *Stratiotes aloides Aquatic Botany* **51**: 269–279.

Smolders A, Roelofs JGM. 1993. Sulphate-mediated iron limitation and eutrophication in aquatic ecosystems. *Aquatic Botany* **46**: 247–253.

Søndergaard M, Sand-Jensen K. 1979. Carbon uptake by leaves and roots of *Litorella uniflora* (L.) Aschers. *Aquatic Botany* **6**: 1–12.

St-Cyr L, Fortin D, Campbell PGC. 1993. Microscopic observations of the iron plaque of a submerged aquatic plant (*Vallisneria americana* Michx). *Aquatic Botany* **46**: 155–167.

Taylor GJ, Crowder AA, Rodden R. 1984. Formation and morphology of an iron plaque on the roots of *Typha latifolia* L. grown in solution culture. *American Journal of Botany* **71**: 666–675.

Tessenow U, Baynes Y. 1978. Redox-chemical effects of *Isoetes lacustrius* L. in littoral sediments of Lake Feldsee. *Archiv für Hydrobiologie* **82**: 20–48.

Yoshida S, Tadano T. 1978. Adaptation of plants to submerged soils. In: Jung GA, ed. *Crop Tolerance to Suboptimal Land Conditions*. Madison, WI, USA: ASA, CSSA, 233–256.

Wium-Andersen S, Andersen JM. 1972. The influence of vegetation on the redox profile of the sediment of Grane Lanso, a Danish *Lobelia* lake. *Limnology and Oceanography* **17**: 948–952.